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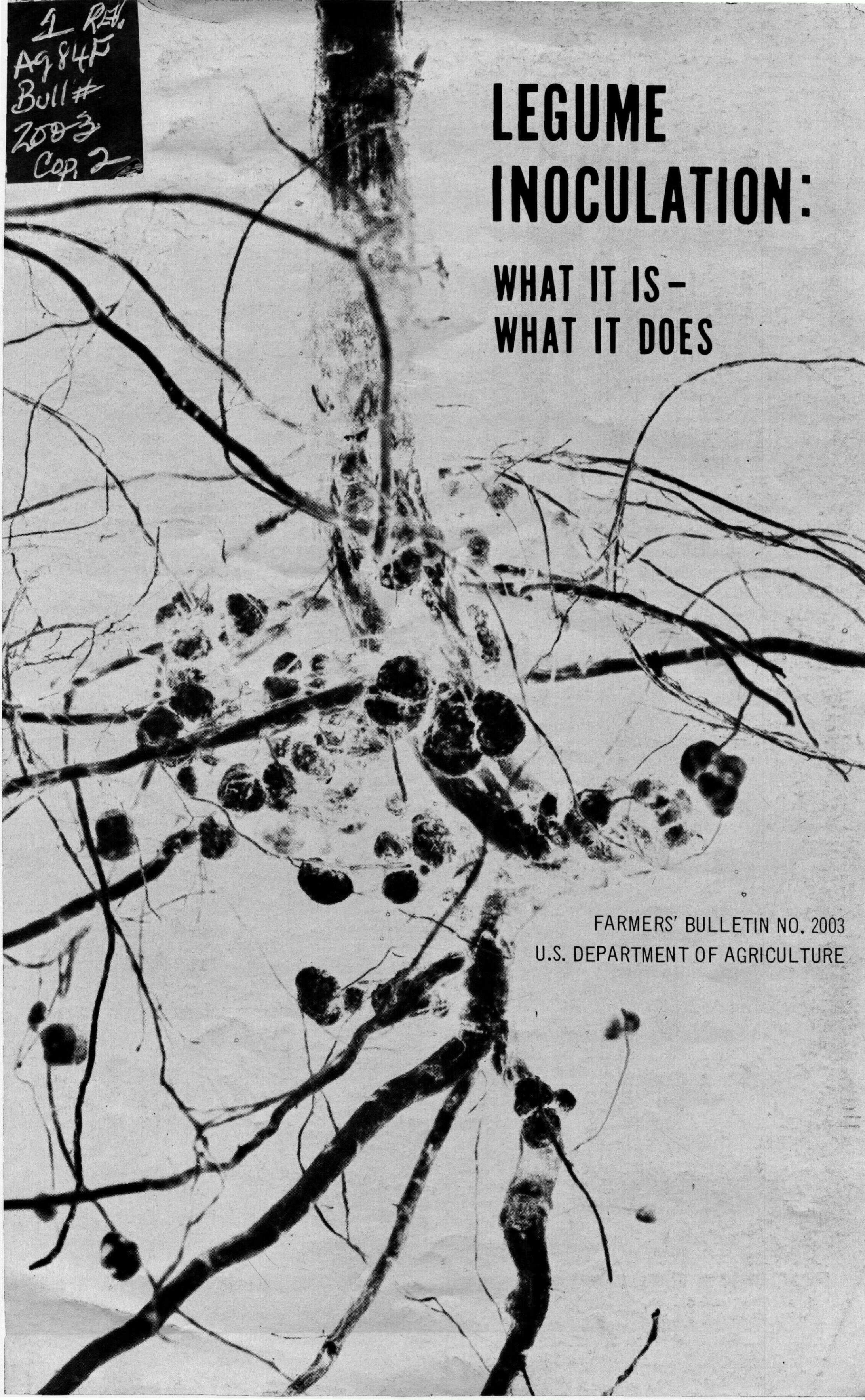
LEGUME INOCULATION:

WHAT IT IS–
WHAT IT DOES



FARMERS' BULLETIN NO. 2003
U.S. DEPARTMENT OF AGRICULTURE

For Successful Legume Inoculation—

Use the right inoculant for the legume.
Keep commercial culture in cool, dark place until used.
Follow directions and mix culture well with seed.
Plant seed within 48 hours after they are inoculated, or reinoculate.
Inoculate in all cases of doubt and always on new land.
Prepare a good, well-fertilized, moist seedbed; after planting small seeds, cultipack the soil.

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BN–27932

Cover illustration.—Highly effective nodulation on soybean roots. Clusters around the taproot were produced by inoculant added to seed.

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LEGUME INOCULATION: WHAT IT IS—WHAT IT DOES

By LEWIS W. ERDMAN, *research microbiologist, Soil and Water Conservation Research Division, Agricultural Research Service* [1]

Inoculation of legumes means the introduction of legume bacteria into the soil to enable the plants to *fix*, or change into usable form, atmospheric nitrogen.

Nitrogen is indispensable to life because it is the key ingredient in protein.

The air we breathe is primarily a mixture of nitrogen and oxygen gases. About 80 percent by volume is pure nitrogen in a free, or uncombined, state. Every acre of land surface has about 35,000 tons of this free nitrogen above it.

Free nitrogen, as such, is useless to plant or animal life. To become useful, it must combine with other elements. But it does not combine with other elements merely by coming in contact with them. It is forced into combination with these elements by powerful influences such as lightning or by bacteria growing on the roots of leguminous plants.

Nitrogen Fixation With Legumes

Farmers can obtain atmospheric nitrogen for their crops by growing inoculated legumes. The inoculating process consists of mixing legume seeds with the correct strain of bacteria culture before the seeds are planted. After the seeds are planted, the bacteria multiply greatly in the soil.

[1] Retired September 1965.

Soon after the legumes begin to grow, the legume bacteria invade the root hairs. The legumes form growths on the roots called nodules. The bacteria live in these nodules and do their beneficial work (fig. 1).

A definite partnership is established. The legume plant furnishes the necessary sugar, or energy. The bacteria use this energy to change the free nitrogen of the atmosphere into a form that the plant can assimilate and use to build protein. The nitrogen is then said to be *fixed*.

Extent of Nitrogen Fixation

The quantity of nitrogen taken from the air and fixed by the legume bacteria for different legumes is difficult to calculate. It varies with (1) the kind of legume, (2) the effectiveness of the legume bacteria, (3) the soil conditions, and (4) the presence of necessary plant-food elements exclusive of nitrogen.

Estimates of the average per acre amounts of nitrogen fixed by bacteria in roots of the most important legumes are given below.

Legume:	*Average amount of nitrogen fixed per acre (pounds)*
Ladino clover	200
Alfalfa	186
Blue lupine	151
Alsike clover	136
Red clover	132
Sweetclover	125
White clover	118
Velvetbean	113
Bur-clover	107
Pastures with legumes	106
Lentils	103

Sourclover	98
Kudzu	88
Crimson clover	85
Lespedeza	85
Fenugreek	82
Vetch	82
Cowpeas	75
Peas	69
Austrian winter pea	54
Soybeans	51
Peanut	42
Beans	40

In high-fertility soils, well supplied with available nitrate nitrogen, little or no fixation may occur. Best results from legume inoculation are obtained on soils of average or very low fertility.

Nitrogen fixation with legumes is estimated to fix more than 4 million tons of atmospheric nitrogen yearly in the United States. Legumes are the chief source of nitrogen added to soils, and even in areas where cheap nitrogen fertilizer is available, nitrogen fixed by legumes plays a conspicuous economic role in modern agriculture.

The Legumes

Legumes are plants that bear their seeds in pods, such as beans and peas. They are used for seed, hay, silage, winter cover crops, and pasture.

Of the more than 12,000 known species of legumes, only about 200 are cultivated. In the United States only about 50 species are grown commercially. Species are further divided into varieties. For instance, more than 100 named soybean varieties are grown in this country.

The legumes are rich in high-quality protein. They are well supplied with phosphorus and calcium. They are a good source of vitamins, especially vitamins A and D. These qualities make legumes one of man's best foods, and almost indispensable for efficient, economical livestock feeding.

The protein in legumes is directly related to high nitrogen content. In this respect they differ markedly

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Figure 1.—Soybean plant on left was grown from uninoculated seed. Other plants were grown from inoculated seed.

from grasses and other nonlegumes. For example, the average protein content of 1 ton of each of eight legume hays was compared with the protein in eight grasses. The legumes averaged 304 pounds of protein per ton and the grasses 156 pounds.

Need for Inoculation

For legume plants to function normally, fix nitrogen, increase yield, and improve the soil, it is necessary to have the proper kind of effective legume bacteria in the soil. Many soils normally do not have sufficient numbers of these bacteria. The purpose of legume inoculation is to compensate for this deficiency.

There is no simple test to determine whether a given soil has sufficient numbers of the right kind of effective legume bacteria to produce maximum benefit to the plants. Farmers who have been growing legumes in soils for long periods generally know whether it is necessary to inoculate legume seeds. On the other hand, many farmers are not sure whether it pays to inoculate. Certainly there is every reason to believe that in many sections of the country thousands of acres of land need effective strains of legume bacteria to produce maximum benefits from leguminous crops.

Legume Bacteria

Legume bacteria are single-celled micro-organisms that vary in size and shape with age, and with the composition of the medium in which they grow. They may be either the usual rod forms, 0.5 to 0.9 micron wide and 1.2 to 3.0 microns long (a micron is $\frac{1}{25,000}$ inch), or the irregular, club-shaped forms.

Unfortunately not all legume bacteria are beneficial. There are some parasitic strains that form nodules on the roots but fail to fix nitrogen. Therefore, the mere number of nodules does not always indicate the value of the inoculant. Effectiveness would have to be measured by evaluating other growth responses, such as mass, vigor, and color, as well as the number of nodules. A deeper, darker green in inoculated legumes is a sure sign of nitrogen fixation by the bacteria.

The best bacteria for legume inoculation are those that compete with the native bacteria for nodule sites on the young plant roots. If the strains used in the inoculant are not competitive, the farmer will soon notice that he is not getting the results he should. Some progress has been made in identifying these competitive bacteria. Such positive identification would help insure beneficial results.

Nodules and Nitrogen

Inoculated legumes growing in normal soils display definite characteristic types of nodule formation. Some of these types are shown in figure 2.

The complete absence of nodules (root on right in fig. 3) indicates that the plant derives all of its nitrogen from the soil. Evidence of nodules (root on left in fig. 3) is a sign that the plant benefited from nitrogen fixed. In fields previously cropped to the same legume, nodules found on the lateral roots are assumed to be formed by the native bacteria. Nodules clustered all around the taproot (fig. 3) are formed by the bacteria from the added culture. If the inside of a nodule is pink or red, this indicates high nitrogen-fixing activity. If white, green, or brown, little or no nitrogen is being fixed.

Inoculated legumes grown with grass contribute to the nitrogen nutrition of the grass. Sometimes this may be observed in the field by the deeper green color of the grass. One experiment showed that clover plants released 72 lb./acre

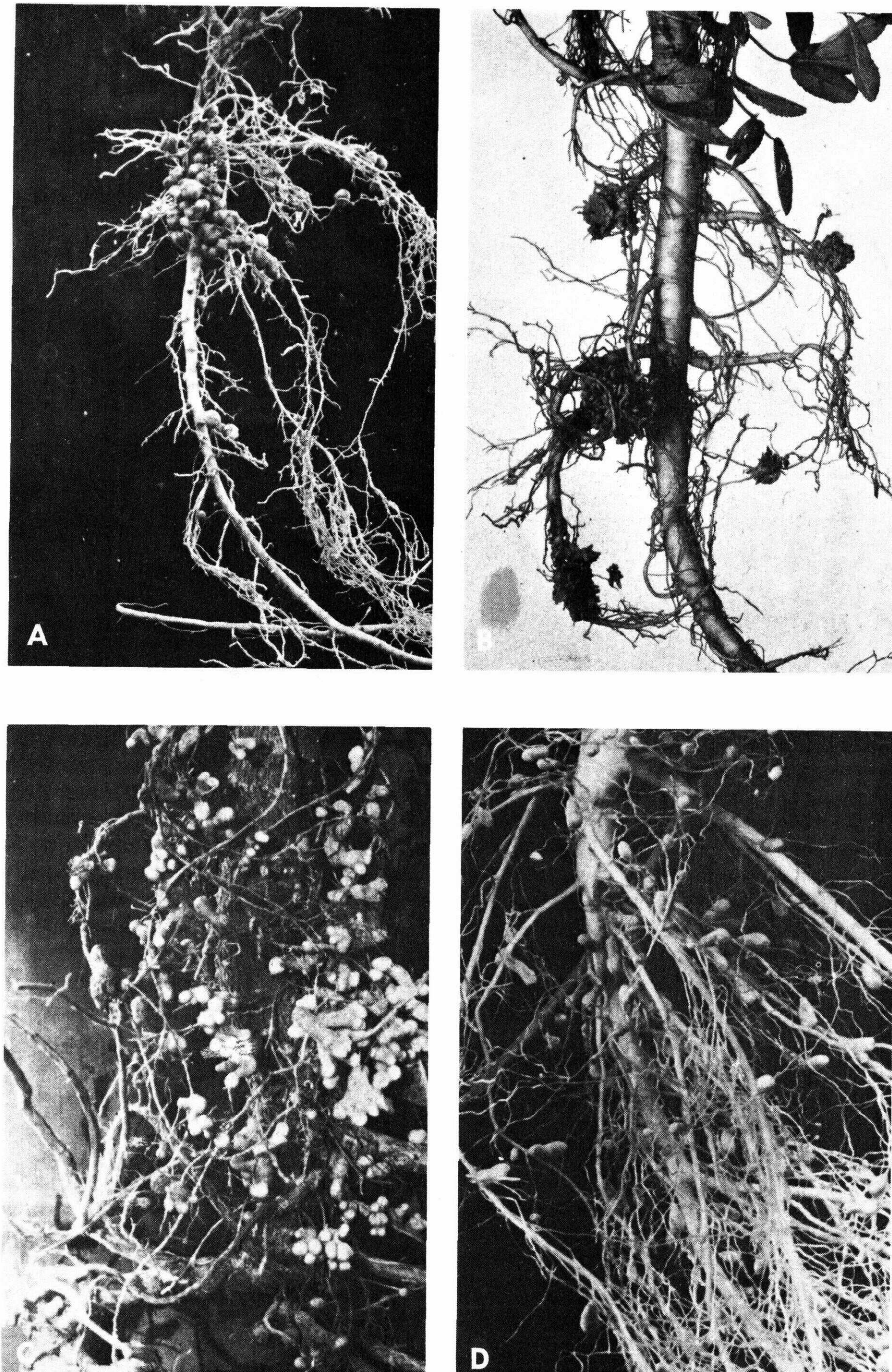

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Figure 2.—Types of nodulation produced by effective strains of bacteria on different legumes: ***A,* Lespedeza; *B,* alfalfa; *C,* black locust; *D,* crimson clover.**

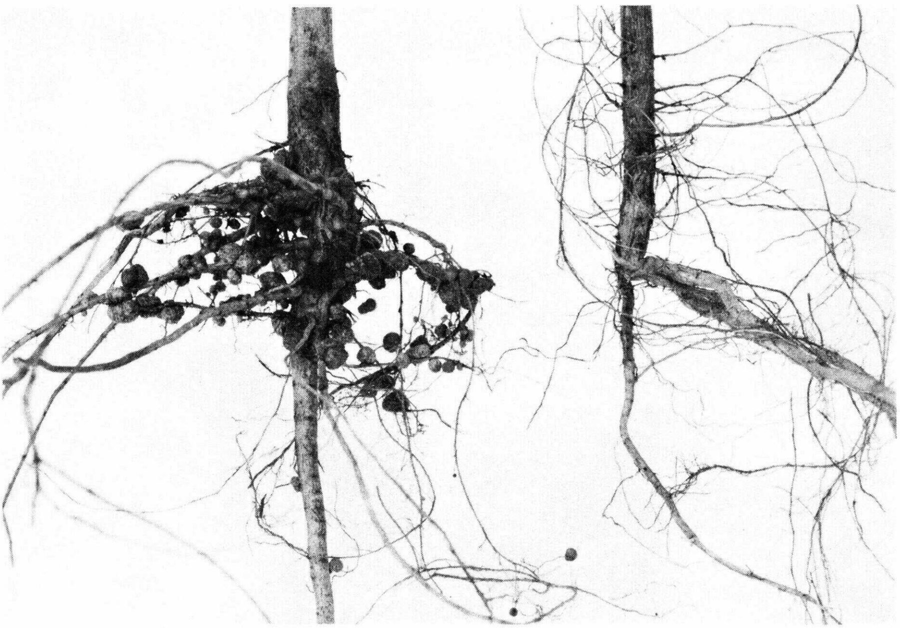

Figure 3.—Nodulated and nonnodulated soybean roots.

of nitrogen, some of which was absorbed by the grass and some by the clover plants.

Generally, as the legumes mature, the nitrogen compounds formed in the nodules furnish nitrogen for building proteins in the leaves, stems, and seeds. In the early stages the nodules may contain 5 to 8 percent of nitrogen, but at seed maturity they are no richer in nitrogen than the rest of the root. The nodules disintegrate rapidly at the time of seed formation.

Bacteria in the Soil

As the nodules slough off the legume plant roots and decompose, many of the bacteria return to the soil. If the chemical reaction of the soil is favorable, if sufficient moisture and plant food are available, and if temperatures are not too high, these bacteria establish themselves in the soil. Continued growing of the same legume in a soil tends to build up the native population of the legume bacteria. However, their persistence in the soil may be lowered by unfavorable soil conditions, such as acidity, low fertility, and the presence of antibiotic substances. Maintaining pH at the right level, between 5.5 and 7, and inoculating each year will go a long way toward keeping nitrogen supplies adequate.[2]

Legume Bacteria Cultures

It is well known that legume bacteria are of different kinds. For example, the bacteria that work on alfalfa and sweetclover will not function on the clovers, nor on peas, beans, soybeans, and other legumes. Conversely, the clover bacteria fail to work on alfalfa and sweetclover.

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[2] pH is a measure of the degree of acidity or alkalinity, with the pH scale ranging from 1 to 14. For further explanation of pH, see Farmers' Bulletin 2124, "Liming Soils: An Aid to Better Farming"; available from U.S. Department of Agriculture, Washington, D.C. 20250. Send your request on a post card. Include your ZIP Code in your return address.

Group Designations

The fact that the legume bacteria are so definitely selective was responsible for the recognition of the so-called cross-inoculation groups of leguminous plants. Any plant within such a group can be inoculated with a culture of the right kind of bacteria. This is usually prepared from several strains known to inoculate effectively all the legumes in that particular group.

The main cross-inoculation culture groups are given below, with a list of the most important legumes in each.

ALFALFA GROUP

Common name	Scientific name
Alfalfa	*Medicago sativa*
Buttonclover	*M. orbicularis*
California bur-clover	*M. denticulata*
Spotted bur-clover	*M. arabica*
Black medic	*M. lupulina*
Snail bur-clover	*M. scutellata*
Tubercle bur-clover	*M. tuberculata*
Little bur-clover	*M. minima*
Tifton bur-clover	*M. rigidula*
Yellow alfalfa	*M. falcata*
White sweetclover	*Melilotus alba*
Hubam sweetclover	*M. alba annua*
Yellow sweetclover	*M. officinalis*
Bitterclover (sourclover)	*M. indica*
Fenugreek	*Trigonella foenum-graceum*

CLOVER GROUP

Common name	Scientific name
Alsike clover	*Trifolium hybridum*
Crimson clover	*T. incarnatum*
Hop clover	*T. agrarium*
Small hop clover	*T. dubium*
Large hop clover	*T. procumbens*
Rabbitfoot clover	*T. arvense*
Red clover	*T. pratense*
White clover	*T. repens*
Ladino clover	*T. repens (giganteum)*
Sub clover	*T. subterraneum*
Strawberry clover	*T. fragiferum*
Berseem clover	*T. alexandrinum*
Cluster clover	*T. glomeratum*
Zigzag clover	*T. medium*
Ball clover	*T. nigrescens*
Persian clover	*T. resupinatum*
Carolina clover	*T. carolinianum*
Rose clover	*T. hirtum*
Buffalo clover	*T. reflexum*
Hungarian clover	*T. pannonicum*
Seaside clover	*T. wormskjoldii*

CLOVER GROUP—Continued

Common name	Scientific name
Lappa clover	*T. lappaceum*
Bigflower clover	*T. michelianum*
Puff clover	*T. fucatum*

PEA AND VETCH GROUP

Field pea	*Pisum arvense*
Garden pea	*P. sativum*
Austrian winter pea	*P. sativum* (var. *arvense*)
Common vetch	*Vicia sativa*
Hairy vetch	*V. villosa*
Horsebean	*V. faba*
Narrowleaf vetch	*V. angustifolia*
Purple vetch	*V. atropurpurea*
Monantha vetch	*V. articulata*
Sweet pea	*Lathyrus odoratus*
Rough pea	*L. hirsutus*
Tangier pea	*L. tingitanus*
Flat pea	*L. sylvestris*
Lentil	*Lens culinaris (esculenta)*

COWPEA GROUP

Cowpea	*Vigna sinensis*
Asparagus-bean	*V. sesquipedalis*
Korean lespedeza	*Lespedeza stipulacea*
Common lespedeza	*L. striata*
Sericea lespedeza	*L. cuneata*
Slender bushclover	*L. virginica*
Bush lespedeza	*L. cyrtobotrya*
Striped crotalaria	*Crotalaria mucronata*
Curara pea	*C. usaramoensis*
Sunn crotalaria	*C. juncea*
Winged crotalaria	*C. sagittalis*
(No common name)	*C. anagyroides*
Florida beggarweed	*Desmodium tortuosum*
Hoary tickclover	*D. canescens*
Bundleflower	*Desmanthus illinoensis*
Kudzu	*Pueraria thunbergiana*
Alyceclover	*Alysicarpus vaginalis*
(No common name)	*Erythrina indica*
Coral tree	*E. varigata*
Pigeonpea	*Cajanus cajan (indicus)*
Guar	*Cyamopsis tetragonoloba*
Jackbean	*Canavalia ensiformis*
Peanut	*Arachis hypogaea*
Velvetbean	*Stizolobium deeringianum*
Lima bean	*Phaseolus lunatus (macrocarpus)*
Adzuki bean	*P. angularis*
Mat bean	*P. aconitifolius*
Mung bean	*P. aureus*

COWPEA GROUP—Continued

Common name	Scientific name
Tepary bean	*P. acutifolius* (var. *latifolius*)
Black gram	*Chamaecrista fasciculata*
Rice bean	*P. mungo*
Partridge-pea	*P. calcaratus*
Hairy indigo	*Indigofera hirsuta*

BEAN GROUP

Garden beans, kidney bean, Navy bean, wax bean, Pinto bean.	*Phaseolus vulgaris*
Scarlet runner bean	*P. coccineus* (*multiflorus*)

LUPINE GROUP

Blue lupine	*Lupinus angustifolius*
Yellow lupine	*L. luteus*
White lupine	*L. albus*
Washington lupine	*L. polyphyllus*
Sundial	*L. perennis*
Texas bluebonnet	*L. subcarnosus*
Serradella	*Ornithopus sativus*

SOYBEAN GROUP

All varieties of soybeans.	*Glycine max* (*Soja max*)

It is obviously not necessary to have a specific culture of legume bacteria for every legume to be planted. It is necessary, however, to have a sufficient number of specified effective strains in an inoculant to inoculate a number of different legumes.

The following legumes appear to require specific strains of legume bacteria for effective inoculation:

SPECIFIC STRAIN GROUP

Common name	Scientific name
Birdsfoot trefoil	*Lotus corniculatus*
Big trefoil	*L. uliginosus*
Foxtail dalea	*Dalea alopecuroides*
Black locust	*Robinia pseudoacacia*
Trailing wild bean	*Strophostylea helvola*
Hemp sesbania	*Sesbania exaltata*
Kura clover	*Trifolium ambiguum*
Sanfoin	*Onobrychis vulgaris* (*sativus*)
Crown vetch	*Coronilla varia*

SPECIFIC STRAIN GROUP—Continued

Common name	Scientific name
Siberian pea-shrub	*Caragana arborescens*
Garbanzo	*Cicer arietinum*
Leadplant	*Amorpha canescens*
Acacia	*Acacia linifolia*
Kangaroo-thorn	*A. armata*
Green wattle	*A. decurrens*
Wild-indigo	*Baptisia tinctoria*
Lead tree (Kao Haole)	*Leucaena glauca*
Tropical kudzu	*Pueraria phaseoloides* (*javanica*)
(No common name)	*Centrosema pubescens*
Indigo bush	*Amorpha fruticosa*
Genge	*Astralagus sinicus*
(No common name)	*A. cicer*
(No common name)	*Calopogonium mucunoides*
(No common name)	*Tephrosia candida*
(No common name)	*T. vogelii*

It has been known for a long time that nodules do not form on some legumes. Among these are redbud (*Cercis canadensis*), Kentucky coffeetree (*Gymnocladus dioicus*), honeylocust (*Gleditsia triacanthos*), and sicklepod (*Cassia tora*).

Strain Variation

Some legume varieties, particularly soybeans and peas, have specific bacteria preferences. A strain that produces excellent results on one variety may be a poor nitrogen fixer on another variety.

There is also variation in effectiveness between strains of bacteria isolated from the same legumes and from different legumes within the same group. This type of strain variation among the legume bacteria has great practical significance. As nitrogen fixers, some are good, some are bad, and others are mediocre.

The search for new and better strains is continuous. Strain variation among legume bacteria is illustrated in figure 4.

These observations should be emphasized:

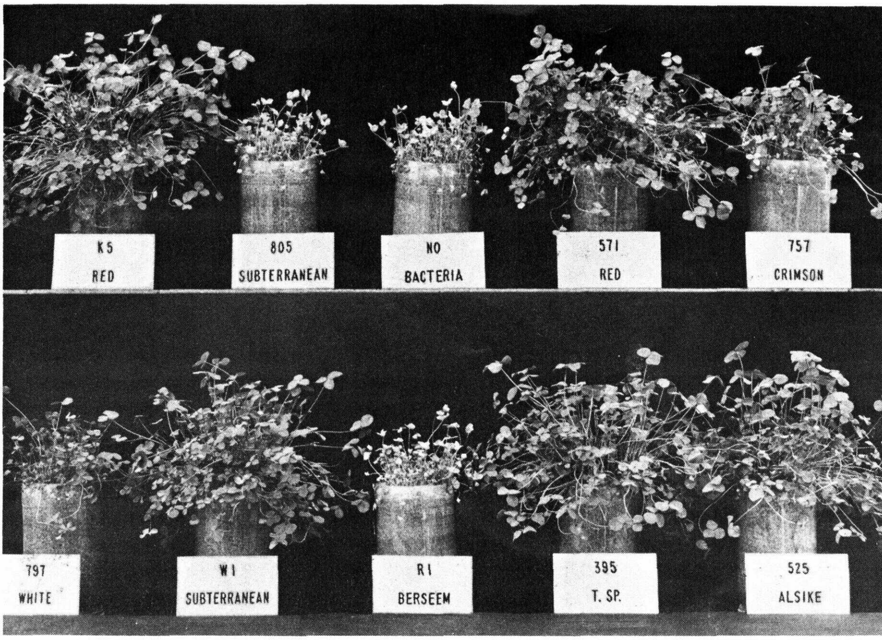

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Figure 4.—How different strains of clover bacteria affect Ladino clover.

1. The alfalfa and sweetclover strains work on alfalfa or sweetclover equally well, but they fail to produce nitrogen fixation in bur-clovers and fenugreek. Strains from bur-clover and fenugreek, on the other hand, work and fix nitrogen on bur-clovers, fenugreek, alfalfa, and sweetclovers.

2. Strains from red and white clovers fix nitrogen on their host plants, but not all of them effectively inoculate crimson clover. One strain isolated from berseem clover was effective on all clovers tested except the white and the red.

3. Strains of legume bacteria show definite varietal preferences. For example, some soybean bacteria work on one or two soybean varieties better than on others. The same is true for different varieties of canning or freezing peas.

4. Strains of legume bacteria from birdsfoot trefoil may be highly effective on their host, but totally ineffective on big trefoil, which is another species. Strains that are highly effective on big trefoil may not work on birdsfoot trefoil. However, experimental work has shown that when effective strains for each of these trefoils are mixed together into a single inoculant, both species can be satisfactorily inoculated.

Greenhouse tests of different strains of legume bacteria enable the bacteriologist to select the best strains for a given legume. Field tests of these selected strains are then desirable to see how they affect the legumes when they are grown on the farm (fig. 5).

Inoculation Methods

Farmers have a choice of several methods for inoculating their legumes.

The "On the Farm" Method

Inoculants are purchased from the seed supplier. Be sure to get the

right kind of bacteria for the specific seed to be planted and be sure that the culture is within the expiration date. Keep in a cool place until ready to use. Follow directions on the package. Water or a sugar solution is usually recommended for mixing the culture with the seed, which should be planted as soon as possible.

Water causes the inoculants to stick to the seeds better than when inoculants are used without water. Some farmers prefer to mix the inoculant in the drill box without water. However, if a given quantity of a soybean inoculant is satisfactory when applied without water, the same quantity would be equally satisfactory for up to five times as much soybean seed if applied with water. For red clover, inoculant requirements are cut approximately in half by using inoculants mixed with water.

Custom Inoculation

This is a service performed by retail seed dealers, grain elevators, or seed companies, who use machines especially adapted for mixing seeds with inoculants. Usually as in the case of soybeans, farmers bring their seed in to be cleaned and inoculated. Good results may be expected if certain precautions are observed. The inoculant used must be viable and contain an abundance of effective legume bacteria. The bacteria are supplied in liquid or a frozen concentrate which is handy for use in mixing machines. High temperatures should be avoided. The machines must be clean and free of chemicals used for seed treatment—this is important. Sow inoculated seed as quickly as possible after treatment.

Use of Additives or Adhesives

Shortly before a rain is the ideal time for planting inoculated seed. If inoculated small legume seeds remain on or near the surface of the soil, exposed to drying winds for several weeks, supplemental inoculation is advisable. This may be done by mixing a legume inoculant with cottonseed meal or wheat middlings or even sand, and broadcast-

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Figure 5.—How soybeans respond to soybean bacteria: *A*, Uninoculated; *B*, inoculated.

CHEMICAL SEED TREATMENTS

Chemical seed treatments to kill fungi, injurious soil organisms, and insects should not be confused with seed inoculation. *Most seed disinfectants are toxic to legume bacteria*, but some of the bacteria have a degree of tolerance to the organic compounds if certain precautions are taken. Sometimes seeds are treated to protect them against harmful bacteria and fungi. Such treated seeds can be inoculated by making a paste with the inoculant and mixing this thoroughly with the seed. If the seeds are planted within 2 hours, successful inoculation may follow. If necessary to hold seeds longer, they should be reinoculated. Complete fertilizers should not be allowed to come in direct contact with inoculated legume seeds. Phosphates are not as harmful as nitrogen or potassium materials. Basic slag or ground limestone are actually beneficial and are sometimes used to pellet the inoculated legume seed.

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ing the mixture over the soil immediately before or after a rain.

Planting inoculated seeds in dry soil is not recommended. However, if an adhesive, like sirup or one of the commercial preparations is used to mix the inoculant with the seed, the life of the bacteria can sometimes be maintained for 2 to 3 weeks. When a 10-percent sirup solution was used on alfalfa seed planted in dry soil in September in North Carolina, 1,915 pounds of dry hay were obtained from an experimental plot. When water alone was used under the same conditions, only 1,040 pounds of hay were harvested. If rain does not come before the 2- to 3-week period has elapsed, it is always advisable to reinoculate the soil.

Preinoculated Seeds

With this method the legume seeds are treated with various forms of legume cultures under patented processes. The farmer pays an extra premium for this type of inoculation, but he does not have to do any of the inoculation—he buys the seeds ready to plant.

USE OF LEGUME INOCULANTS

A survey of the legume inoculant industry made in 1963 showed that the total number of commercial cultures (bushel-size units) used in that year was around 24,776,000. Of this amount 1,253,996 bushels of alfalfa and clover seeds and 1,304,990 bushels of soybeans were preinoculated. About 75 percent of the total cultures were applied to soybeans; nearly 15 percent were used on alfalfa and clover seeds; and 6.5 percent on peas and vetch. The demand for the other groups was considerably less. It was estimated that farmers spent about $7,413,000 for legume inoculants in 1963.

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